BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

V:(W 9/30

INTERLIBRARY LOAN FORM

Examiner: Ralph Gitomer
Telephone Number: 308-0732
Serial Number: 9/868,200
Please attach copy of abstract, citation or bibliography, if available
ONLY ONE REQUEST PER FORM

Art Unit: 1651
Date Requested:9/30/03
Date Needed: sometime

Hay E. D.
Interaction of Migrating Embryonic Cells with Extracellular Matrix
Experimental Biology and Medicine Vol. 10 1985 174-193
ISSN: 0071-3384

PTO Call No. _____ Accession Number 466325

SCIENTIFIC LIBRARY USE ONLY

LIBRARY ACTION	LC	NAL	NIH	NLM	NBS	PTO	OTHER
DATE							
INITIALS							
RESULTS							
EXAMINER CALLED							
PAGE COUNT							
MONEY SPENT							

Expl Biol. Med., vol. 10, pp. 174–193 (Karger, Basel 1985)

Interaction of Migrating Embryonic Cells with Extracellular Matrix

Elizabeth D. Hay

Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Mass., USA

Introduction

Cell migrations in the embryo begin at gastrulation. For the higher vertebrate (bird, mammal), this is a period of time marked by the appearance of tracts of extracellular matrix (ECM) between the newly created epiblast and hypoblast of the germ disc [*Critchley* et al., 1979; *Hay,* 1981]. While there are undoubtedly movements of cells within the epithelial sheets comprising the epiblast and hypoblast, the first dramatic cell migration begins with the formation of the primitive streak in the midline of the epiblast (fig. 1). Here, newly forming mesenchymal cells leave the epiblast, lose epithelial polarity [*Stern,* 1982], develop pseudopodia and filopodia, and move through the ECM, which is loosely organized [*Hay,* 1968] and probably rich in hyaluronic acid (HA). Cells attach to the basal laminae of the epiblast and hypoblast, which they move along, and to each other. Bands of fibronectin (FN) associated with such basal laminae may play a role in contact guidance [*Critchley* et al., 1979]. In the area of the primitive streak, cell junctions are well developed and seem to provide a kind of contact inhibition of movement that encourages the now actively motile cells to move away from the primitive streak [*Hay,* 1968].

In the sea urchin, gastrulation is accomplished both by epithelial infolding (to form the archenteron) and by cell migration (primary mesenchyme). The primary mesenchymal cells arise in the region of the infolding archenteron and migrate along FN-rich basal laminae, for which they now develop a new affinity [*Katow* et al., 1982; *Fink and McClay,* 1985]. Affinity for their former epithelial neighbors is lost and new cell surface antigens are expressed [*Fink and McClay,* 1985]. Video tapes reveal that isolated primary

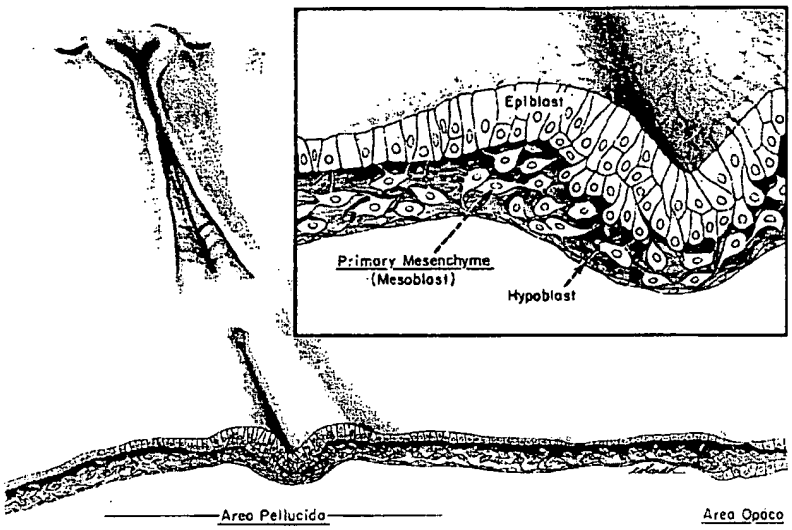


Fig. 1. Drawing illustrating a plane of section through the primitive streak of a stage 8 chick embryo [from *Hay,* 1968].

mesenchymal cells move in vitro on FN at a speed of 1–5 μm/min and on plain culture dishes at an average of less than 1 μm/min [*Venkatasubramanian and Solursh,* 1984]. Synthesis of sulfated molecules that might attach the cells to ECM is correlated with cell spreading and migration [*Karp and Solursh,* 1974].

While most of the evidence for it is indirect, the idea that the composition and disposition of ECM also plays a vital role in other embryonic cell migrations is widely accepted. In addition to its presumed role in gastrulation, FN is associated with primordial germ cell [*Heasman* et al., 1981] and neural crest [*Rosavio* et al., 1983] movement. In vitro, FN is chemotactic [*Kleinman* et al., 1981]. FN-rich ECM in 3D array is the only migratory substratum that permits neural crest cells to achieve a forward speed as great as 50 μm/h in vitro [*Rosavio* et al., 1983]. FN is found in fibrils as well as basal laminae in the early embryo [*Mayer* et al., 1981] and is in a good position to serve as a 3D migratory substratum. The spaces between such fibrils are large and probably filled with HA. There is a good correlation between increased HA content and the ability of ECM to promote cell migration

[*Toole,* 1981]. Moreover, adding HA to collagen gel has been shown to cause endocardial cushion mesenchyme to move faster [*Bernanke and Markwald,* 1982]. There is also a good correlation between cessation of cell migration and loss of FN [*Le Douarin,* 1984], appearance of hyaluronidase, and disappearance of HA [*Toole,* 1981], especially in the developing avian cornea, an organ which we will now discuss in detail.

Corneal Fibroblast Migration in vivo and in vitro

The chicken cornea appears on the 3rd day of incubation between the surface ectoderm and the newly invaginated lens. The primary, or first formed, corneal stroma is produced by the surface ectoderm, which hypertrophies at 3 days and becomes rich in secretory organelles [*Hay and Revel,* 1969; *Dodson and Hay,* 1974]. This first formed stroma consists of approximately 25 layers of collagen fibrils in orthogonal array. The corneal fibroblasts will subsequently migrate on these layers to create a similar number of cell layers with the same orientation as the original collagen [*Trelstad and Coulombre,* 1971]. An endothelium derives from neural crest on the 4th day and between the 5th and 6th day (fig. 2), neural crest derived mesenchymal cells invade the stroma to form the fibroblasts [see *Hay,* 1980].

The invasion of the fibroblasts is correlated with important changes in the composition of the corneal ECM. The primary avian cornea consists of striated fibrils that contain collagen type I and II [*Hendrix* et al., 1982], type IX [*Svoboda* et al., 1985], and probably type V [*Linsenmayer* et al., 1983]. Just prior to its invasion by the fibroblasts, the stroma swells and dramatically increases its HA content [*Toole,* 1981]. During the fibroblast invasion, FN appears for the first time [*Kurkinen* et al., 1979]. Endothelium is the source of the HA, and fibroblasts presumably of the FN [see *Hay,* 1980].

The corneal stroma remains swollen and rich in HA and FN during the next week as the fibroblasts continue to move into it (stages 30–35, fig. 3). They are loosely connected by gap junctions [*Hasty and Hay,* 1977]. On the 12th day, cell division and migration cease, hyaluronidase appears, and HA begins to disappear [see *Toole,* 1981]. By the 14th day (stage 40, fig. 3), stromal condensation can be seen proceeding from posterior to anterior cornea, FN is disappearing, and metachromasia is increasing due to accumulation of corneal sulfated proteoglycans [see *Hay,* 1980]. At hatching (fig. 3), the cornea is completely transparent, HA and FN are gone, and the immobilized fibroblasts are producing abundant collagen and proteoglycan.

Fig. 2. L
of neural cre
epi = epitheli

. shown to cause
· *and Markwald,*
ıf cell migration
nidase, and dis-
ıg avian cornea,

:ion between the
ɔrimary, or first
m, which hyper-
. [*Hay and Revel,*
ısists of approxi-
he corneal fibro-
ı similar number
gen [*Trelstad and*
st on the 4th day
ed mesenchymal
:0].
ɔrtant changes in
ornea consists of
et al., 1982], type
yer et al., 1983].
ells and dramati-
ɔroblast invasion,
ıdothelium is the
:e *Hay,* 1980].
ıd FN during the
;es 30–35, fig. 3).
ay, 1977]. On the
appears, and HA
(stage 40, fig. 3),
terior to anterior
ıcreasing due to
980]. At hatching
are gone, and the
ınd proteoglycan.

Fig. 2. Light micrograph of a section of a stage 27 chick embryonic cornea. Fibroblasts of neural crest origin are beginning migration into the swollen corneal stroma (arrow). epi = epithelium; end = endothelium. Bar = 25 μm [from *Bard and Hay,* 1975].

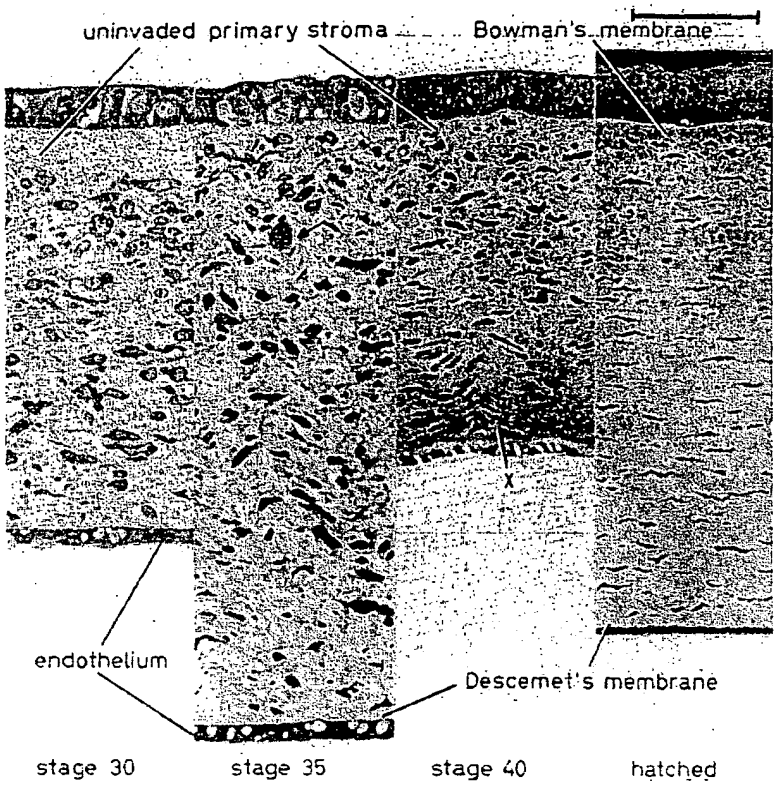
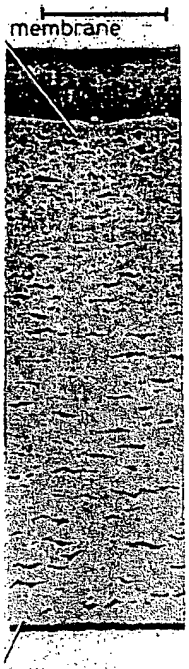


Fig. 3. Light micrographs showing the changes that take place in the embryonic chick cornea between stage 30 and hatching. Condensation of the stroma begins posteriorly (X = stage 40. Bar = 50 μm [from *Hay and Revel,* 1969].

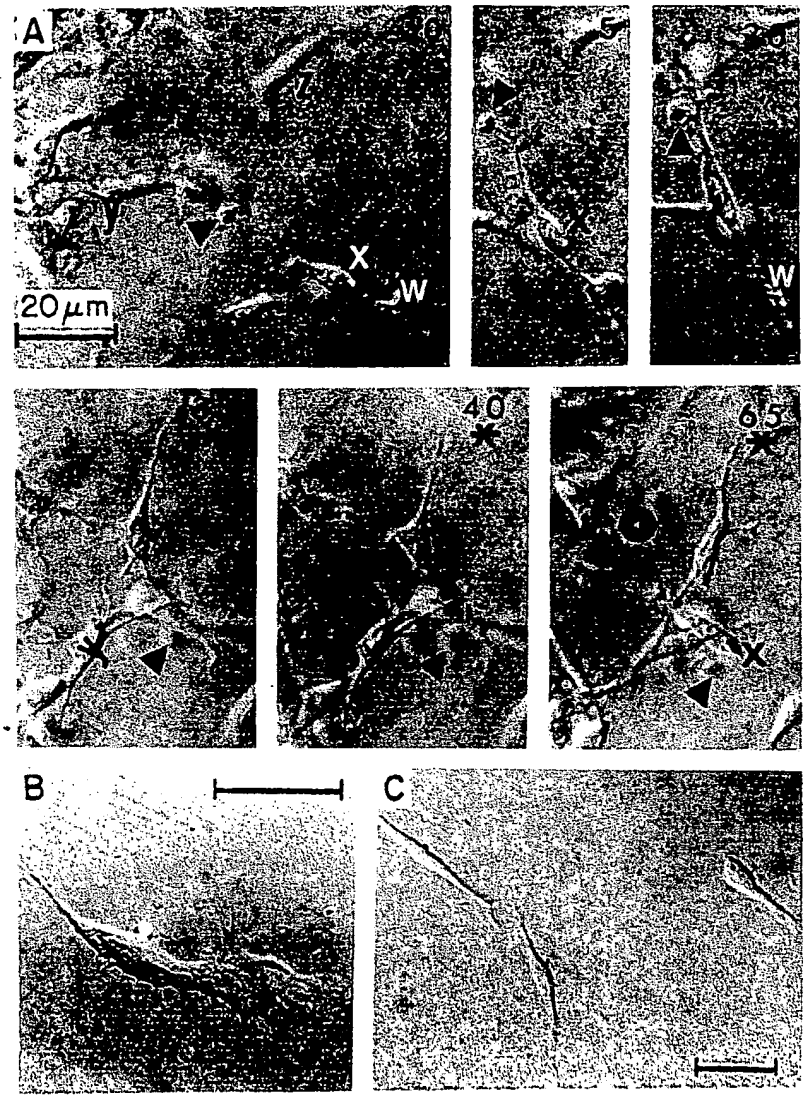
Fig. 4. Light micrographs of living corneal fibroblasts. *A* These cells were filmed in the stage 28 cornea for 0–65 min. The 5- and 30-min photographs are in a slightly different focus plane than 35–65 min (*). Arrowhead indicates a fixed particle for reference. Circle indicates cell process that retracted between 40 and 65 min. The progress of cells W, X, Y, Z is described in the text. *B* Corneal fibroblast migrating on glass. *C* Corneal fibroblasts migrating in collagen gel. Bars = 20 μm. (from *Bard and Hay,* 1975].



hatched

ı the embryonic chick
na begins posteriorly

ells were filmed in the
slightly different focus
:rence. Circle indicates
W, X, Y, Z is describ-
blasts migrating in col-



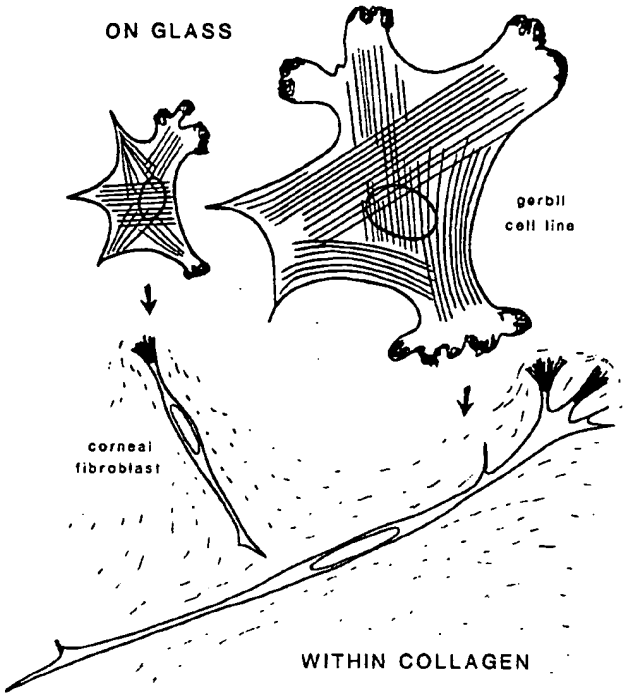
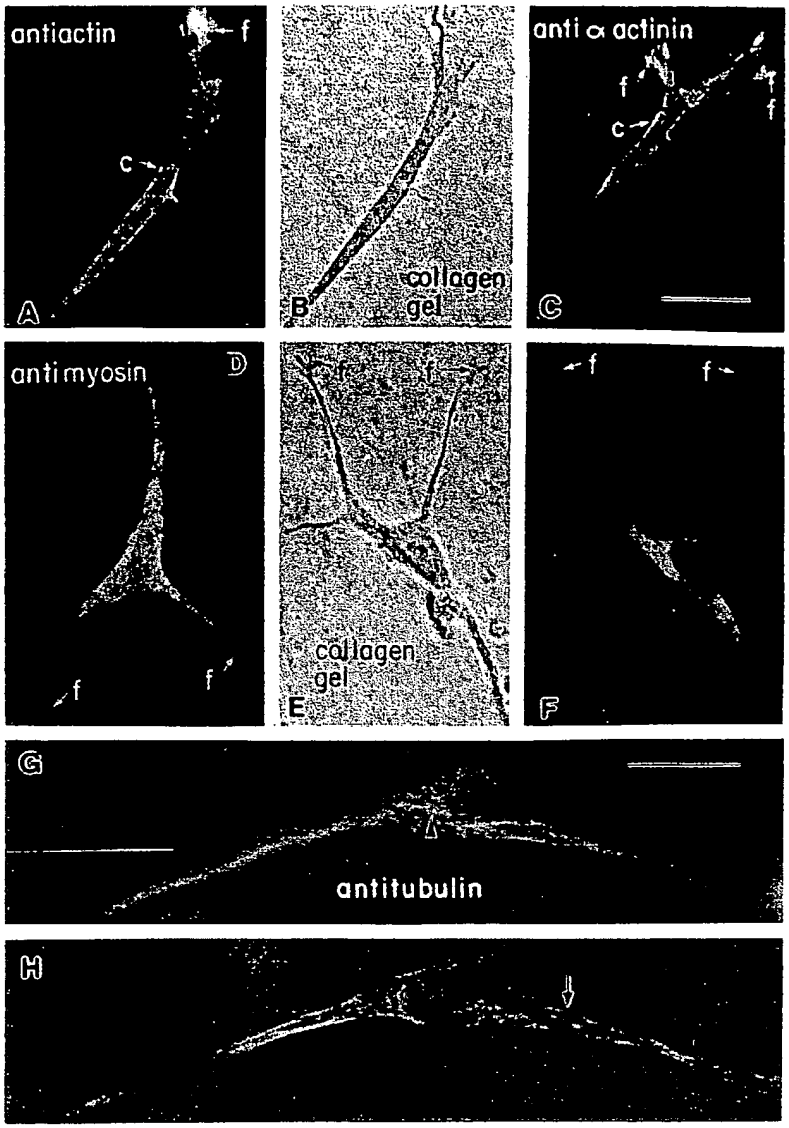


Fig. 5. Diagrams depicting the shape of fibroblasts from embryonic cornea and gerbil fibroma grown on planar substratum (top) and within collagen gel (bottom) [from *Hay,* 1985].

Fig. 6. Demonstration by immunofluorescence of the cytoskeleton of 10-day-old embryonic chick corneal fibroblasts moving in collagen gel. *A* Antiactin stains filopodia (f) and cell cortex (c) intensely. *B* Phase contrast photograph of the cell on the left. *C* Antialpha-actinin pattern of staining is similar to that of actin. *D* Antimyosin does not stain the filopodia (f). *E* Phase contrast photograph of the gerbil fibroma cell shown in *F*. *F* Antimyosin does not stain filopodia (f) of this fibroblast either. *G* Antitubulin stains microtubules and the microtubule organizing center (arrowhead). *H* Same cell at another plane of focus. Arrow points to a microtubule. Bars = 20 μm. *A–F* from *Tomasek* et al. [1982]; *G–H* from *Tomasek and Hay* [1984].



ınic cornea and gerbil
ɔm) [from *Hay*, 1985].

ı of 10-day-old embry-
s filopodia (f) and cell
't. *C* Antialpha-actinin
ain the filopodia (f). *E*
imyosin does not stain
:s and the microtubule
ıs. Arrow points to a
om *Tomasek und Hay*

At the time of initial invasion by the fibroblasts (fig. 2), the acellular avian cornea is thin enough (stroma, 50 μm thick, epithelium 20 μm, and endothelium, 5 μm that one can visualize the cells in whole mounts using Nomarski optics. *Bard and Hay* [1975] excised intact 6-day-old chick corneas and filmed the invading fibroblasts in a Dvorak chamber for up to 4 h. In the sequence shown in figure 4A, an elongate fibroblast (X), which has just divided and is separating from daughter (W), is moving toward the particle labeled by the arrowhead. Fibroblasts (Y) and (Z) are moving toward each other in a plane below the level of focus of this photograph. Five minutes later, (5, fig. 4), moving at a rate of 1 μm/min, (X) has reached the particle and touches it with filopodia on its leading pseudopodium. By 30 min, (X) is passing the particle. The movement involves a steady flow forward of the leading pseudopodium and cell body along the surrounding collagen fibrils (not visualized by light microscopy). The trailing pseudopodium becomes attenuated (30, fig. 4) and finally lets go of stroma, or in this case, of cell (w). The trailing process rapidly retracts to the cell body so that the moving cell is temporarily shaped like a pear (X, 65, fig. 4).

Corneal fibroblasts on glass, (fig. 4B) or suspended within collagen gel (fig. 4C) move at the same rate (fig. 4A) and also retract the trailing edge from time to time to permit forward progress [*Bard and Hay,* 1975]. However, on a planar substratum, the fibroblast is flattened, due presumably to strong substratum adhesion, and exhibits a ruffled border on its leading edge (fig. 4B).

Contact inhibition of movement plays an important role in directing the fibroblasts towards the unoccupied middle of the corneal stroma. The periphery is crowded (fig. 2) and cell processes that contact neighbors freeze, causing the cells to create new cell extensions on other surfaces [*Bard and Hay,* 1975]. The phenomenon is similar to that observed in vitro on planar substrata [*Abercrombie,* 1967], but had not previously been described in vivo. The time frames 35–65 are at a different plane (*) than time frame 0 in figure 4 and shows cells (Y) and (Z) to good advantage. In the frame 35* (fig. 4), the leading filopodia on cell (Y) and (Z) are about to touch. When they make contact (40*, fig. 4), they freeze (contact inhibition). Fifteen minutes later (65*, fig. 4), cell (Z) is moving away from the contact zone using another pseudopodium (upper right, 65*, fig. 4). During this interval, cell (Z) also made contact with a cell on the left (circle, 65*, fig. 4) and retracted the cell process involved. In the meantime, cell (X) is passing above the contact inhibited cells (compare frame 65* with frame 0, fig. 4). Fibroblasts cultured within collagen gels show the same type of contact inhibition [*Bard and Hay,* 1975].

2), the acellular
1 20 μm, and en-
ounts using No-
d chick corneas
or up to 4 h. In
), which has just
vard the particle
ing toward each
ih. Five minutes
:hed the particle
3y 30 min, (X) is
forward of the
3 collagen fibrils
iodium becomes
this case, of cell
that the moving

thin collagen gel
the trailing edge
y, 1975]. Howev-
e presumably to
:r on its leading

e in directing the
eal stroma. The
neighbors freeze,
es [*Bard and Hay*,
1 planar substrata
in vivo. The time
0 in figure 4 and
(fig. 4), the lead-
hey make contact
later (65*, fig. 4),
er pseudopodium
ide contact with a
is involved. In the
s (compare frame
gen gels show the

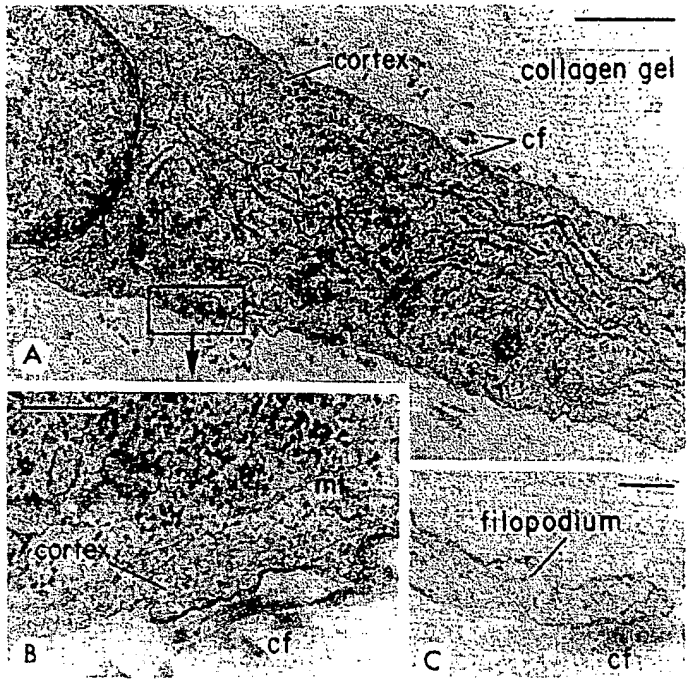


Fig. 7. Electron micrographs of fibroblasts cultured in collagen gels. *A* Collagen fibrils (cf) are closely associated with the surface of the elongated fibroblast. *B* An area of cell cortex similar to that enclosed in the rectangle in *A*. Microtubules (mt) appear to insert in the actin-rich cortex. *C* A filopodium contacting a collagen fibril (cf) contains fine microfilaments. Bars = 100 nm [from *Tomasek* et al., 1982].

The highly elongate shape of the fibroblast moving in ECM (fig. 4A, C) contrasts with the flattened morphology of cells grown on planar substrata (fig. 4B) (also see chapters by previous authors in this volume). Indeed, as adhesion to plastic or glass increases, cells develop prominent stress fibers (fig. 5) that actually seem to impede their migration [*Herman* et al., 1981]. We have recently investigated the structure of the cytoskeleton in the elongated fibroblast moving within ECM, and in the next section we will draw comparisons with cytoskeletal morphology illustrated elsewhere in this volume for cells grown on planar substrata.

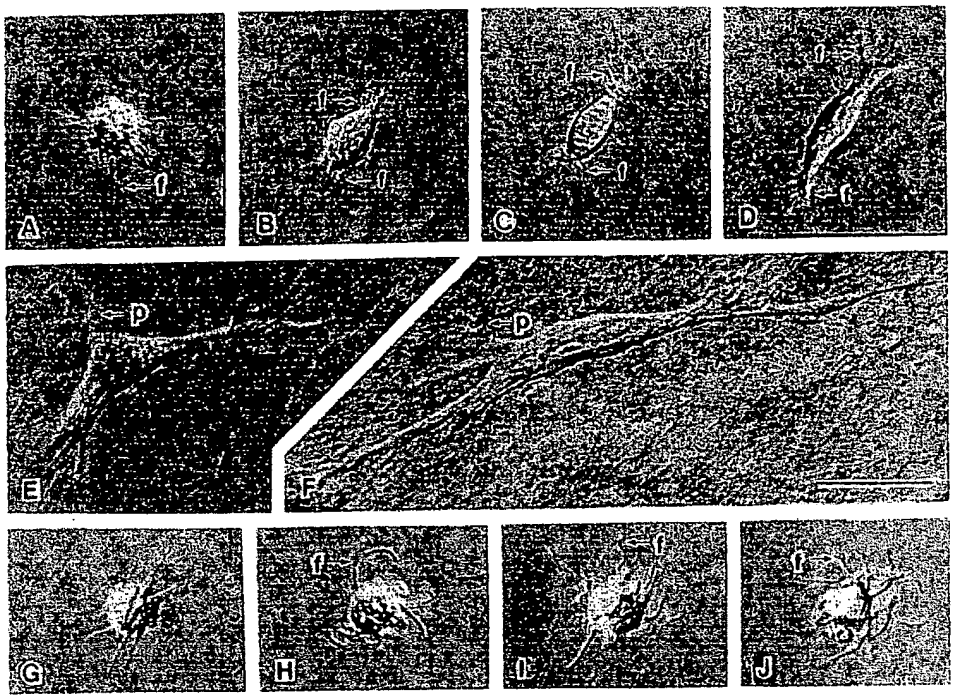
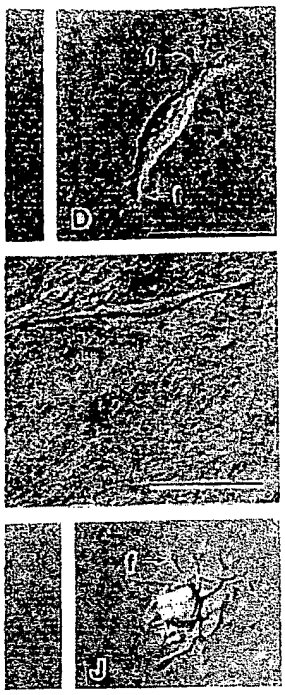
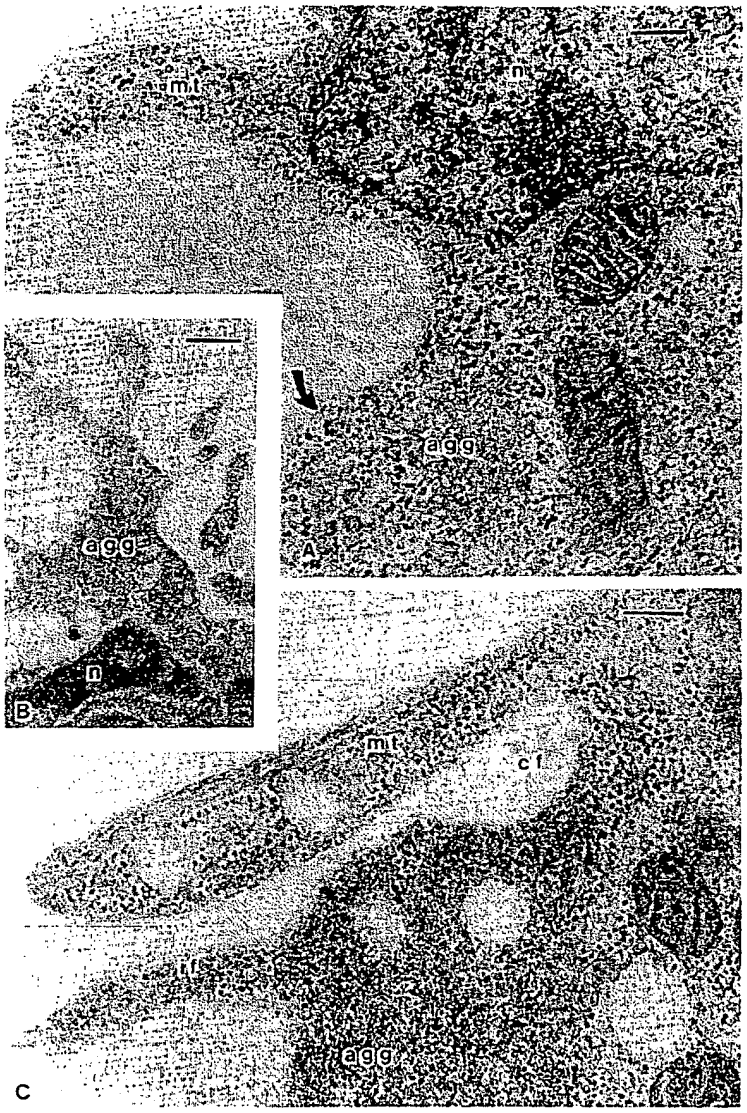


Fig. 8. Light micrographs of corneal fibroblasts cultured in collagen gel for 30 min *(A)*, 1 h *(G–J)*, 2 h *(B, C)*, 3 h *(D)*, 6 h *(E)*, and 18 h *(F)*. *A–F* show the normal time course of cell elongation in collagen gel described in the text. *G* is a vehicular control for cytochalasin D treatment *(H–J)*. *H* was treated with 2 μ*M* of the drug, *I* with 4 μ*M*, and *J* with 6 μM for 1 h from the beginning of the culture period. f = filopodia; p = cell process on the side of the cell. Bar = 20 μm [from *Tomasek and Hay*, 1984].

Fig. 9. Electron micrographs of corneal fibroblasts cultured in collagen gel and treated with 2 *M* cytochalasin D for 1 h from the beginning of culture. *A* Microtubules (mt) are present in cell body and aberrant filopodia. agg = actin aggregate; n = nucleus. Arrow points to former location of the actin cell cortex. *B* Actin aggregate (agg) extends from the cell body into aberrant filopodia. *C* Intermediate filaments (if) are present in the filopodium shown in the lower left of this electron micrograph. cf = collagen fibril in the gel. Bars: *A*, *C* 200 nm; *B* 500 nm [from *Tomasek and Hay*, 1984].



n gel for 30 min *(A)*,
al time course of cell
l for cytochalasin D
J with 6 μM for 1 h
n the side of the cell.



agen gel and treated
ubules (mt) are pres-
eus. Arrow points to
om the cell body into
odium shown in the
A, *C* 200 nm; *B* 500

Role of Cytoskeleton in Fibroblast Elongation and Migration

In the work to be reported in this section, fibroblasts were removed from 10-day-old chicken corneas by trypsin-collagenase digestion and grown in collagen gels thin enough to permit good optical resolution of the cell by phase contrast or Nomarski optics and the cytoskeleton by immunofluorescence [*Tomasek* et al., 1982; *Tomasek and Hay,* 1984]. When stained with antibodies to actin, filopodia (f, fig. 6A) show an intense reaction. The cell cortex, (c, fig. 6A) also reacts strongly with the antibody, whereas the cytosol shows diffuse staining. Alpha-actinin (fig. 6C) shows the same distribution as actin. Myosin, on the other hand, is detectable only in the cytosol (fig. 6D–F). Filopodia show no reaction with antimyosin. Microtubules course the length of the cell (fig. 6G, H). Although these same cells develop stress fibers when cultured on glass or plastic [*Tomasek* et al., 1982; fig. 5], no stress fibers are observed in corneal fibroblasts grown in collagen gels.

Electron microscopy confirms the absence of stress fibers in fibroblasts grown in collagen gels [*Tomasek* et al., 1982]. The cells make close contact with surrounding collagen fibrils, but do not develop the adhesion plaques present in cells growing on planar substrata. The actin-rich cortex and filopodia contain the expected meshwork of fine filaments (fig. 7). Microtubules do not extend into the filopodia. They seem to insert into the actin-rich cortex (fig. 7B).

Evidence that the actin-rich cortex interacts with ECM was obtained by studying the effect of cytoskeletal-disrupting drugs on corneal fibroblast elongation in collagen gels [*Tomasek and Hay,* 1984]. The first step in elongation is the projection of filopodia by the enzyme-isolated, rounded cell (fig. 8A) shortly after suspension in the gel. Contact with collagen fibrils seems essential for this step; fibroblasts suspended in methylcellulose do not project filopodia [*Tomasek and Hay,* 1984]. If the rounded cells in the gel are treated with cytochalasin D from the beginning of the culture period, the filopodia (f, fig. 8H–J) that are extended are long and floppy. Electron microscopy reveals the content of these abnormal filopodia to be highly variable. They may contain ribosomes and microtubules (mt, fig. 9A, C), intermediate filaments (if, fig. 9C), or actin aggregates (agg, fig. 9B). Electron microscopy confirms the immunofluorescent demonstration [*Tomasek and Hay,* 1984] that cytochalasin has indeed aggregated the cell's actin (agg, fig. 9A–C) and disrupted the cell cortex (arrow, fig. 9A).

The second step in elongation is the transposition, within the first 2 h of culture, of the filopodia to opposite ends of the rounded fibroblast (fig. 8B, C), and the third step is extension of pseudopodia in the direction of the filopodia (fig. 8D). By 6 h (fig. 8E), the fibroblast is distinctly bipolar in shape. Cells treated with cytochalasin at the beginning of the culture remain round. Cells treated after assuming the bipolar shape remain bipolar, but are clumpy in appearance, due to aggregation of actin [*Tomasek and Hay,* 1984]. They do not elongate any further. Such fibroblasts are unable to extend cell processes or migrate in the gel [*Hay,* unpubl. observations].

The fourth step in elongation involves further extension of the pseudopodia and takes over 12 h to complete (fig. 8F). Not surprisingly, this step requires microtubules. If bipolar cells (fig. 8E) are treated with nocodazole or taxol, they do not elongate further [*Tomasek and Hay,* 1984]. Although they extend and retract filopodia and pseudopodia, bipolar fibroblasts with disrupted microtubules make no forward progress within the collagen gel [*Hay,* unpubl. observations]. Interestingly, neither nocodazole nor taxol interfere with filopodial extension and assumption of the bipolar shape. However, cytochalasin D prevents bipolar cells from achieving the highly elongate final shape. Thus, both intact actin and intact microtubules are required for final elongation.

We conclude that interaction of the actin-rich cell cortex with ECM is required for, and is sufficient for, extension of filopodia, capping of filopodia to opposite ends of the fibroblast, and assumption of the bipolar shape [*Tomasek and Hay,* 1984]. Interaction of the actin cortex with the surrounding collagen fibrils continues to be required for the final elongation step, but microtubules that seem to insert into the cortex bring about the final elongation. Fibroblasts treated with cytochalasin, nocodazole, or taxol cannot make forward progress in the collagen gel.

Mechanisms of Cell-Matrix Interaction during Cell Migration

In this section, we will speculate first on the possible manner in which the actin-rich cortex of fibroblasts might interact with ECM and then on the possible role of cytoskeletal-ECM interaction in cell migration within collagen gels. Considerable evidence is accumulating for the idea that cells possess in their plasmalemmas a variety of receptors for ECM molecules [reviewed by *Hay,* 1985]. A putative receptor for FN with a molecular weight of 140 kd has recently been isolated from osteosarcoma cells [*Pytela*

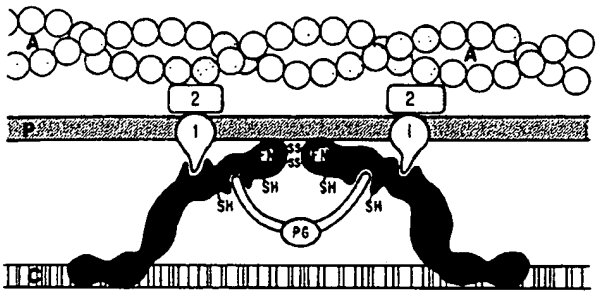


Fig. 10. Speculative diagram depicting putative receptors (1) and accessory proteins (2) linking intracellular actin (A) across the plasmalemma (P) with extracellular fibronectin (FN). FN, in turn, binds to proteoglycan (PG) and collagen (C) [from *Hynes and Yamada,* 1982].

et al., 1985]. A membrane-intercalated glycoprotein of this size could bind FN extracellularly, span the plasmalemma, and bind inside the cell to actin or to intermediate actin-binding proteins (fig. 10). Evidence that the fibroblast cytoskeleton coaligns with underlying FN has been obtained from in vitro studies of cells grown on planar substrata [*Hynes and Yamada,* 1982]. It is tempting to believe that the fibroblast in corneal stroma or collagen gel uses FN receptors to attach its actin-rich cell cortex to the surrounding ECM and thereby change its shape in response to ECM. In vivo, FN appears in the developing cornea at the time of fibroblast invasion [*Kurkinen* et al., 1979], and fibroblasts are notorious for their production of FN [*Hynes and Yamada,* 1982]. While the fibroblast might contain receptors for other ECM molecules [see *Hay,* 1985], the bulk of evidence available today (see Introduction) implicates interaction with FN (attached or not attached to collagen fibrils) in mesenchymal cell migration.

How might interaction of the actin cytoskeleton with FN-rich matrices be involved in cell migration? While we have learned a great deal in recent years about muscle motility, the role of actin and myosin in nonmuscle motility is still poorly understood. Even fibroblasts cultured on planar substrata possess an actin cortex, and it has been proposed that myosin in the cytosol generates the force that propels the cytoplasm forward by interacting with this structurally stable actin-plasmalemma complex [*Willingham* et al., 1981a, b; *Tomasek* et al., 1982]. The cytosol flows forward toward an area of apparent membrane instability, which is characterized by a ruffling membrane in the case of cells on planar substrata or by abundant filopodia

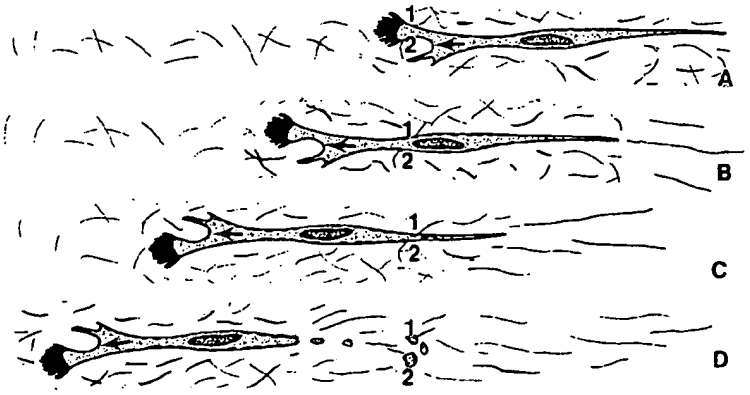


Fig. 11. A new hypothesis is presented in the text to explain how a stable complex of actin cortex-plasmalemma-ECM could be compatible with cell locomotion in ECM. The leading end of the cell is composed of actin-rich, myosin-poor filopodia actively exploring ECM [*Tomasek* et al., 1982]. The hypothesis speculates that this is the area where new plasmalemma and actin cortex are generated that establish stable interactions with ECM (labeled 1, 2 in *A*). The active filopodial region moves ahead, propelled by the forward-flowing cytosol behind it, leaving these actin complexes behind (1, 2 in *B*). After further cytoplasmic flow forward, the first-formed actin complexes (1, 2 in *C*) are located in the trailing end of the cell. When the trailing cell process retracts, remnants of the cell (1, 2 in *D*) are left behind in the stroma. All the time, the forward movement is generated by interaction of the myosin-rich cytosol (arrows) with the fixed actin cortex. The hypothesis would explain the apparent movement backwards of particles fixed to both the top and the bottom of fibroblasts on planar substrata [*Harris*, 1973].

in the case of cells moving in ECM. Ruffled membranes seem to represent aberrant filopodia [*Tomasek* et al., 1982].

This myosin-free leading edge could be a region in which new actin complexes and plasmalemma are generated to permit the forward translocation (fig. 11). Within ECM, it may be supposed that the leading end of the cell explores the surrounding substratum, attaches preferentially to FN, continues to generate new membrane anteriorly, and leaves behind stable FN-actin complexes on the cell surface. The role of the ECM would be to stabilize the actin-plasmalemma complex all around the cell so that the myosin-rich cytosol can flow forward past it. The attachment to ECM is so strong that collagen fibrils can be pulled along by the cell [*Harris* et al., 1981]. When the cell moves its rear end forward, it must break these old contacts with the matrix, and when it does (fig. 11D), bits of cytoplasm are

left in its wake. The so-called particles (arrowheads, fig. 4) observed within corneal stroma are probably just such pieces of cytoplasm.

The hypothesis that the cortex of the front end of the moving cell becomes the cortex of the rear end (fig. 11) is novel, but it explains better than existing hypotheses what is known about cell migration within ECM. All surfaces, except the leading edge, of the elongate cell are morphologically equivalent (figs. 6, 7). The so-called cytosol, which today is viewed as a relatively insoluble cytomatrix that interacts with cell corganelles [*Porter* et al., 1983], is clearly moving forward at the steady rate of 1 μm/min (fig. 4). If the actin cortex were to move forward also, it would not be possible for a sliding interaction between actin and myosin to produce a force that would propel the cell forward. Such a force could be generated by interaction of the myosin-rich cytomatrix with a fixed actin cortex. A cardinal feature of our hypothesis is that the myosin-rich cytomatrix containing the nucleus is moving in the same general direction (forward) everywhere, along both new (arrow fig. 11A) and old (1, 2, fig. 11B, C), actin cortex. The fact that this idea has not emerged from numerous studies of cell migration in vitro [see *Harris,* 1973] can probably be explained by the abnormal morphology of the cell on planar substrata (fig. 5). The cell appears to use only its bottom surface to move on the substratum. However, the 'front end becomes the rear end' hypothesis (fig. 11) could explain many puzzling features of fibroblast migration on planar substrata, such as the apparent movement backwards of particles on both the top and bottom of the cell [*Harris,* 1973].

While actin-myosin interaction has received the most mention in theories of cell locomotion, microtubules are also involved. In the case of cells grown on planar substrata, microtubules seem to be essential for directional movement [*Vasilev and Gelfand,* 1976]. In this chapter, we report that cells in collagen gels do not move forward without microtubules. We suggest that microtubule assembly is affected by ECM via the actin cortex. The major role of the microtubules is to create and maintain the highly elongate cell shape, which in turn may somehow guide the direction of flow of the cytosol during cell locomotion.

We have much to learn about the way ECM promotes and guides cell migrations in the embryo. The case of the developing cornea discussed in this chapter provides one of the simplest situations for further study along these lines. Here, a cell-free stroma suddenly swells with HA and invites the neural crest cells waiting in its periphery to move into its newly FN-rich interstices. Add to the theory of cytoskeleton-mediated forward movement in ECM presented above, the phenomenon of contact inhibition in the crowd-

ed periphery, and the picture is almost complete. In areas of cell contact, rising intracellular calcium levels might disrupt actin-myosin interaction in the cell cortex, thus immobilizing cell processes. Inevitably, the cells move toward the center of the cornea. By the 12th day, crowded local conditions somehow bring cell migration to a halt, but 12-day-old fibroblasts transposed to cell-free collagen gel in vitro begin to move again. Hopefully, additional experiments in the future, using both collagen gels and living corneas, will lead to a greater understanding of the way in which mesenchymal cells are guided by and use ECM in their migrations to developmentally significant destinations in the embryo.

Summary

Mesenchymal cells characteristically interact with ECM along their entire cell surface. They exhibit polarity in the sense that they are typically bipolar in shape when migrating, with a leading pseudopodium tipped by small cell processes (filopodia) that explore the ECM, and a trailing end. Suspended in situ within its natural stroma or in vitro in a collagen gel, the embryonic corneal fibroblast moves its cell body and leading pseudopodium forward at a rate of about 1 μm/min; from time to time the trailing part of the cell breaks its ECM contacts and snaps forward, thus allowing the cell to progress in its migration. The movement of a fibroblast suspended in ECM requires that the leading end generate new membrane, new contacts with ECM, and new actin-membrane complexes that are left behind to interact with myosin in the cytosol to create a propelling force that moves the cytoplasm forward. We speculate that the attachment of the cell cortex to ECM is stable while the inner cytoplasm is flowing by; when the rear end breaks away from ECM, bits of the cell are left behind. Both the actin and the microtubular cytoskeleton are involved in cell elongation and migration in ECM as judged by the effects of cytoskeletal disrupting drugs on these processes. The hypothesis is presented that the actin cortex interacts with ECM via transmembrane receptors, and that microtubules interact with the actin cortex. Transposition of the migrating cells in the embryo requires an intact ECM, which by its disposition could play a role in guiding cells to their targets.

References

Abercrombie, M.: Contact inhibition: the phenomenon and its biological implications. Natn. Cancer Inst. Monogr. 26: 249–277 (1967).

Bard, J. B. L; Hay, E. D.: The behaviour of fibroblasts from the developing avian cornea: their morphology and movement in situ and in vitro. J. Cell. Biol. *67:* 400–418 (1975).

Bernanke, D. H.; Markwald, R. R.: Migratory behavior of cardiac cushion tissue cells in a collagen-lattice culture system. Devl. Biol. *91:* 235–245 (1982).

Critchley, D. R.; England, M. A.; Wakely, J.; Hynes, R. O.: Distribution of fibronectin in the ectoderm of gastrulating chick embryos. Nature, Lond. *280:* 498–500 (1979).

Dodson, J.W.; Hay, E.D.: Secretion of collagen by corneal epithelium. II. Effect of the underlying substratum on secretion and polymerization of epithelial products. J. Exp. Zool. *189* 51–72 (1974).

Fink, R.D.; McClay, D.R.: Three cell recognition changes accompany the ingression of sea urchin primary mesenchyme cells. Devl Biol. *107:* 66–74 (1985).

Harris, A.K.: Cell surface movements related to cell locomotion; in Locomotion of tissue cells, pp. 3–26 (Elsevier/Excerpta Medica/North-Holland, Amsterdam 1973).

Harris, A.K.; Stopak, D.; Wild, P.: Fibroblast fraction as a mechanism for collagen morphogenesis. Nature, Lond. *290:* 249–251 (1981).

Hasty, D.L.; Hay, E.D.: Freeze-fracture studies of the developing cell surface. I. The plasmalemma of the corneal fibroblast. J. Cell Biol. *72:* 667–686 (1977).

Hay, E.D.: Organization and fine structure of epithelium and mesenchyme in the developing chick embryo; in Fleischmajer, Billingham, Epithelial-mesenchymal interactions, pp. 53–71 (Williams & Wilkins, Baltimore 1968).

Hay, E.D.: Development of the vertebrate cornea. Int. Rev. Cytol. *63:* 263–322 (1980).

Hay, E.D.: Cell biology of extracellular matrix, pp. 1–417 (Plenum Publishing, New York 1981).

Hay, E.D.: Extracellular matrix, cell polarity and epithelial-mesenchymal transformation; in Edelman, Molecular determinants of animal form (Liss, New York 1985).

Hay, E.D.; Revel, J.-P.: Fine structure of the developing avian cornea. Monogr. devl Biol., vol. 1, pp. 1–144 (Karger, Basel 1969).

Heasman, J.; Hynes, R.O.; Swan, A.P.; Thomas, V.; Wylie, C.C.: Primordial germ cells of *Xenopus* embryos. The role of fibronectin in their adhesion during migration. Cell *27:* 437–447 (1981).

Hendrix, M.J.C.; Hay, E.D.; Mark, K. von der; Linsenmayer, T.F.: Immunohistochemical localization of collagen types I and II in the developing chick cornea by electron microscopy. Investve Ophthal. vis. Sci. *22:* 359–375 (1982).

Herman, I.M.; Crisona, N.J.; Pollard, T.D.: Relation between cell activity and the distribution of cytoplasmic actin and myosin. J. Cell Biol. *90:* 84–91 (1981).

Hynes, R.O.; Yamada, K.M.: Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. *95:* 369–377 (1982).

Karp, G.C.; Solursh, M.: Acid mucopolysaccharide metabolism, the cell surface, and primary mesenchyme cell activity in the sea urchin embryo. Devl Biol. *41:* 110–123 (1974).

Katow, H.; Yamada, K.M.; Solursh, M.: Occurrence of fibronectin on the primary mesenchymal cell surface during migration in the sea urchin embryo. Differentiation *22:* 120–124 (1982).

Kleinmann, H.K.; Klebe, R.J.; Martin, G.R.: Role of collagenous matrices in the adhesion and growth of cells. J. Cell Biol. *88:* 473–485 (1981).

Kurkinen, M.; Alitalo, K.; Vaheri, A.; Stenman, S.; Saxen, L.: Fibronectin in the development of the embryonic chick eye. Devl Biol. *69:* 589–600 (1979).

Le Douarin, N.M.: Cell migrations in embryos. Cell *38:* 353–360 (1984).

Linsenmayer, T.F.; Fitch, J.M.; Zak, N.B.; Gibney, E.; Sanderson, R.D.; Mayne, R.: Monoclonal antibodies against chicken type V collagen: production, specifity, and use for immunocytochemical localization in embryonic cornea and other organs. J. Cell Biol. *96:* 124–132 (1983).

Mayer, B.W.; Hay, E.D.; Hynes, R.O.: Immunocytochemical localization of fibronectin in embryonic chick trunk and area vasculosa. Devl Biol. *82:* 267–286 (1981).

Effect of the under-
ducts. J. Exp. Zool.

ne ingression of sea

ocomotion of tissue
rdam 1973).
or collagen morpho-

urface. I. The plas-
977).
ne in the developing
al interactions, pp.

: 263–322 (1980).
olishing, New York

l transformation; in
ork 1985).
nogr. devl Biol., vol.

ordial germ cells of
migration. Cell 27:

mmunohistochemical
nea by electron mi-

ivity and the distri-
(1981).
ycoproteins. J. Cell

urface, and primary
)–123 (1974).
: primary mesenchy-
tiation 22: 120–124

ices in the adhesion

in the development

)84).
; Mayne, R.: Mono-
fity, and use for im-
ins. J. Cell Biol. 96:

on of fibronectin in
286 (1981).

Porter, K.R.; Beckerle, M.; McNiven, M.: The cytoplasmic matrix; in McIntosh, Modern cell biology, pp. 257–302 (Liss, New York 1983).

Pytela, R.; Pierschbacher, M.D.; Ruoslathi, E.: Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell *40:* 191–198 (1985).

Rosavio, R.A.; Delouvee, A.; Yamada, K.M.; Timpl, R.; Thiery, J.P.: Neural crest cell migration: requirements for exogenous fibronectin and high cell density. J. Cell Biol. *96:* 462–473 (1983).

Stern, C.D.: Localization of the sodium pump in the epiblast of the early chick embryo. J. Anat. *134:* 606–607 (1982).

Svoboda, K.; Parson, J.; Konomi, H.; Hay, E.D.; Olsen, B.R.: Expression of mRNA for types I, II, and IX collagen embryonic avian corneal epithelium (Abstract). J. Cell Biol. (in press, 1985).

Tomasek, J.J.; Hay, E.D.: Analysis of the role of microfilaments and microtubules in acquisition of bipolarity and elongation of fibroblasts in hydrated collagen gels. J. Cell Biol. *99:* 536–549 (1984).

Tomasek, J.J.; Hay, E.D.; Fujiwara, K.: Collagen modulates cell shape and cytoskeleton of embryonic corneal and fibroma fibroblasts: distribution of actin, a-actinin, and myosin. Devl Biol. *92:* 107–122 (1982).

Toole, B.P.: Glycosaminoglycans in morphogenesis; in Hay, Cell biology of extracellular matrix, pp. 259–294 (Plenum Publishing, New York 1981).

Trelstad, R.L.; Coulombre, A.J.: Morphogenesis of the collagenous stroma in the chick cornea. J. Cell Biol. *50:* 840–858 (1971).

Vasilev, J.M.; Gelfand, I.M.: Effect of colcemid on morphogenetic processes and locomotion of fibroblasts; in Goldman, Pollard, Rosenbaum, Cell motility, pp. 247–264 (Cold Spring Harbor Laboratory, New York 1976).

Venkatasubramanian, K.; Solursh, M.: Adhesive and migratory behavior of normal and sulfate-deficient sea urchin cells in vitro. Expl Cell Res. *154:* 421–431 (1984).

Willingham, M.C.; Yamada, S.S.; Bechtel, P.J.; Rutherford, M.A.; Pastan, I.: Ultrastructural immunocytochemical localization of myosin in cultured fibroblastic cells. J. Histochem. Cytochem. *29:* 1289–1301 (1981a).

Willingham, M.C.; Yamada, S.S.; Davies, P.J.A.; Rutherford, A.V.; Gallo, M.G.; Pastan, I.: Intracellular localization of actin in cultured fibroblasts by electron microscopic immunocytochemistry. J. Histochem. Cytochem. *29:* 17–37 (1981b).

Elizabeth D. Hay, MD, Department of Anatomy and Cellular Biology, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115 (USA)